10/758,772

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(FILE 'HOME' ENTERED AT 14:37:26 ON 30 MAY 2006)

FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH, LIFESCI' ENTERED AT 14:37:42 ON 30 MAY 2006

L1 3847 S (WHEY(W)ACIDIC(W)PROTEIN OR WAP OR MMTV OR MOUSE(W)MAMMARY(W)
L2 1078988 S (DNA(W)CONSTRUCT OR VECTOR OR DNA(W)SEQUENCE OR POLYNUCLEOTID
L3 168 S L1(6A)L2
L4 87 DUP REM L3 (81 DUPLICATES REMOVED)

=> d au ti so pi 50-87 l4

L4 ANSWER 50 OF 87 MEDLINE on STN DUPLICATE 6
AU Fan W; Ma J X; Cheng L; Norris J S
TI Molecular cloning of TA16, a transcriptional repressor that may mediate glucocorticoid-induced growth arrest of leiomyosarcoma cells.
SO Molecular endocrinology (Baltimore, Md.), (1997 Aug) Vol. 11, No. 9, pp. 1342-52.
Journal code: 8801431. ISSN: 0888-8809.

L4 ANSWER 51 OF 87 MEDLINE on STN DUPLICATE 7
AU Hoffmann A; Villalba M; Journot L; Spengler D
TI A novel tetracycline-dependent expression vector with low basal expression and potent regulatory properties in various mammalian cell lines.
SO Nucleic acids research, (1997 Mar 1) Vol. 25, No. 5, pp. 1078-9.
Journal code: 0411011. ISSN: 0305-1048.

L4 ANSWER 52 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
IN Guenzburg, Walter H.; Salmons, Brian
TI Viral and plasmid vectors encoding mouse mammary tumor virus Naf repressor or Sag antigen for control of viral infections or lymphocyte gene therapy
SO PCT Int. Appl., 44 pp.
CODEN: PIXXD2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9628564	A1	19960919	WO 1996-EP1002	19960308
W: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9651040	A1	19961002	AU 1996-51040	19960308
EP 817859	A1	19980114	EP 1996-907399	19960308
EP 817859	B1	20020116		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI				
JP 11508441	T2	19990727	JP 1996-527260	19960308
EP 1162273	A1	20011212	EP 2001-118945	19960308
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI				
AT 212061	E	20020215	AT 1996-907399	19960308
ES 2171657	T3	20020916	ES 1996-907399	19960308
US 6730511	B1	20040504	US 1997-925214	19970908
US 2002061297	A1	20020523	US 2001-965135	20010927

L4 ANSWER 53 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
IN Guenzburg, Walter H.; Winder, David; Saller, Robert Michael
TI Vectors carrying therapeutic genes encoding antimicrobial peptides for gene therapy
SO PCT Int. Appl., 54 pp.

CODEN: PIXXD2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9628563	A1	19960919	WO 1996-EP1001	19960308
W: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9651039	A1	19961002	AU 1996-51039	19960308
EP 817858	A1	19980114	EP 1996-907398	19960308
EP 817858	B1	20030423		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI				
JP 11503305	T2	19990326	JP 1996-527259	19960308
AT 238427	E	20030515	AT 1996-907398	19960308
PT 817858	T	20030930	PT 1996-907398	19960308
ES 2198479	T3	20040201	ES 1996-907398	19960308
US 7022319	B1	20060404	US 1997-999690	19970908

L4 ANSWER 54 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
IN Lubron, Henryk; Drohan, William N.; Velander, William H.
TI Transgenic animals expressing genes for human coagulation factor VIII and von willebrand factor with secretion of the protein into milk
SO PCT Int. Appl., 28 pp.
CODEN: PIXXD2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9609377	A1	19960328	WO 1995-US11781	19950921
W: CA, JP, MX				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5880327	A	19990309	US 1994-308518	19940921
CA 2200610	AA	19960328	CA 1995-2200610	19950921
EP 807170	A1	19971119	EP 1995-933128	19950921
EP 807170	B1	20060329		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				
JP 10506013	T2	19980616	JP 1995-510993	19950921
AT 321785	E	20060415	AT 1995-933128	19950921
US 6255554	B1	20010703	US 1999-262017	19990304
US 2002062492	A1	20020523	US 2001-849406	20010507
US 6518482	B2	20030211		

L4 ANSWER 55 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
IN Guenzburg, Walter Henry; Saller, Robert Michael
TI Safe, non-self-inactivating retroviral expression vectors using non-LTR promoters for gene therapy
SO PCT Int. Appl., 40 pp.
CODEN: PIXXD2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9607748	A1	19960314	WO 1995-EP3445	19950901
W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TT, UA, UG, US, UZ, VN				
RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2198210	AA	19960314	CA 1995-2198210	19950901
AU 9535201	A1	19960327	AU 1995-35201	19950901
AU 688590	B2	19980312		
EP 779929	A1	19970625	EP 1995-931969	19950901
EP 779929	B1	20010411		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE

CN 1159210	A	19970910	CN 1995-194903	19950901
CN 1117156	B	20030806		
BR 9508664	A	19980106	BR 1995-8664	19950901
HU 76974	A2	19980128	HU 1997-1764	19950901
HU 221607	B	20021128		
JP 10507628	T2	19980728	JP 1995-509186	19950901
AT 200517	E	20010415	AT 1995-931969	19950901
ES 2156945	T3	20010801	ES 1995-931969	19950901
EE 3492	B1	20010815	EE 1997-40	19950901
PL 184375	B1	20021031	PL 1995-319033	19950901
RU 2199585	C2	20030227	RU 1997-105070	19950901
NO 9700902	A	19970424	NO 1997-902	19970227
NO 317731	B1	20041213		
FI 9700892	A	19970228	FI 1997-892	19970228
HK 1001527	A1	20041126	HK 1998-100508	19980121

L4 ANSWER 56 OF 87 MEDLINE on STN DUPLICATE 8
AU Choong C S; Kemppainen J A; Zhou Z X; Wilson E M
TI Reduced androgen receptor gene expression with first exon CAG repeat expansion.
SO Molecular endocrinology (Baltimore, Md.), (1996 Dec) Vol. 10, No. 12, pp. 1527-35.
Journal code: 8801431. ISSN: 0888-8809.

L4 ANSWER 57 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
AU Asano, T.; Zwelling, L. A.; An, T.; McWatters, A.; Herzog, C. E.; Mayes, J.; Loughlin, S. M.; Kleinerman, E. S.
TI Effect of transfection of a Drosophila topoisomerase II gene into a human brain tumor cell line intrinsically resistant to etoposide
SO British Journal of Cancer (1996), 73(11), 1373-1380
CODEN: BJCAAI; ISSN: 0007-0920

L4 ANSWER 58 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
AU Asano, Takeshi; An, Taeha; Mayes, Janice; Zwelling, Leonard A.; Kleinerman, Eugenie S.
TI Transfection of human topoisomerase IIα into etoposide-resistant cells: transient increase in sensitivity followed by down-regulation of the endogenous gene
SO Biochemical Journal (1996), 319(1), 307-313
CODEN: BIJOAK; ISSN: 0264-6021

L4 ANSWER 59 OF 87 MEDLINE on STN DUPLICATE 9
AU Dolnikov A; King A; Luxford C; Symonds G; Sun L Q
TI Ribozyme-mediated suppression of v-myc expression abrogates apoptosis in transformed monocytes.
SO Cancer gene therapy, (1996 Sep-Oct) Vol. 3, No. 5, pp. 289-95.
Journal code: 9432230. ISSN: 0929-1903.

L4 ANSWER 60 OF 87 MEDLINE on STN DUPLICATE 10
AU Asano T; An T; Zwelling L A; Takano H; Fojo A T; Kleinerman E S
TI Transfection of a human topoisomerase II alpha gene into etoposide-resistant human breast tumor cells sensitizes the cells to etoposide.
SO Oncology research, (1996) Vol. 8, No. 3, pp. 101-10.
Journal code: 9208097. ISSN: 0965-0407.

L4 ANSWER 61 OF 87 LIFESCI COPYRIGHT 2006 CSA on STN
AU Candau, R.; Chavez, S.; Beato, M.*
TI The hormone responsive region of mouse mammary tumor virus positions a nucleosome and precludes access of nuclear factor I to the promoter
SO STEROID BIOCHEM. MOL. BIOL., (1996) vol. 57, no. 1-2, pp. 19-31.
ISSN: 0960-0760.

L4 ANSWER 62 OF 87 MEDLINE on STN DUPLICATE 11
AU Chavez S; Candau R; Truss M; Beato M
TI Constitutive repression and nuclear factor I-dependent hormone activation of the mouse mammary tumor virus promoter in Saccharomyces cerevisiae.
SO Molecular and cellular biology, (1995 Dec) Vol. 15, No. 12, pp. 6987-98.
Journal code: 8109087. ISSN: 0270-7306.

L4 ANSWER 63 OF 87 MEDLINE on STN DUPLICATE 12
AU Minch S L; Kallio P T; Bailey J E
TI Tissue plasminogen activator coexpressed in Chinese hamster ovary cells with alpha(2,6)-sialyltransferase contains NeuAc alpha(2,6)Gal beta(1,4)Glc-N-AcR linkages.
SO Biotechnology progress, (1995 May-Jun) Vol. 11, No. 3, pp. 348-51.
Journal code: 8506292. ISSN: 8756-7938.

L4 ANSWER 64 OF 87 MEDLINE on STN DUPLICATE 13
AU Denman R B; Smedman M; Ju W; Rubenstein R; Potempska A; Miller D L
TI Ribozyme mediated degradation of beta-amyloid peptide precursor mRNA in COS-7 cells.
SO Nucleic acids research, (1994 Jun 25) Vol. 22, No. 12, pp. 2375-82.
Journal code: 0411011. ISSN: 0305-1048.

L4 ANSWER 65 OF 87 MEDLINE on STN DUPLICATE 14
AU Eder J P Jr; Chan V T; Niemierko E; Teicher B A; Schnipper L E
TI Conditional expression of wild-type topoisomerase II complements a mutant enzyme in mammalian cells.
SO The Journal of biological chemistry, (1993 Jul 5) Vol. 268, No. 19, pp. 13844-9.
Journal code: 2985121R. ISSN: 0021-9258.

L4 ANSWER 66 OF 87 MEDLINE on STN DUPLICATE 15
AU Sugimoto Y; Hamada H; Tsukahara S; Noguchi K; Yamaguchi K; Sato M; Tsuruo T
TI Molecular cloning and characterization of the complementary DNA for the M(r) 85,000 protein overexpressed in adriamycin-resistant human tumor cells.
SO Cancer research, (1993 Jun 1) Vol. 53, No. 11, pp. 2538-43.
Journal code: 2984705R. ISSN: 0008-5472.

L4 ANSWER 67 OF 87 MEDLINE on STN DUPLICATE 16
AU Stocklin E; Botteri F; Groner B
TI An activated allele of the c-erbB-2 oncogene impairs kidney and lung function and causes early death of transgenic mice.
SO The Journal of cell biology, (1993 Jul) Vol. 122, No. 1, pp. 199-208.
Journal code: 0375356. ISSN: 0021-9525.

L4 ANSWER 68 OF 87 MEDLINE on STN DUPLICATE 17
AU Habraken Y; Laval F
TI Increased resistance of the Chinese hamster mutant irs1 cells to monofunctional alkylating agents by transfection of the E. coli or mammalian N3-methyladenine-DNA-glycosylase genes.
SO Mutation research, (1993 Mar) Vol. 293, No. 3, pp. 187-95.
Journal code: 0400763. ISSN: 0027-5107.

L4 ANSWER 69 OF 87 MEDLINE on STN DUPLICATE 18
AU Kong C T; Varde A; Lever J E
TI Targeting of recombinant Na+/glucose cotransporter (SGLT1) to the apical membrane.
SO FEBS letters, (1993 Oct 25) Vol. 333, No. 1-2, pp. 1-4.
Journal code: 0155157. ISSN: 0014-5793.

L4 ANSWER 70 OF 87 MEDLINE on STN DUPLICATE 19
AU Hirt R P; Poulain-Godefroy O; Billotte J; Kraehenbuhl J P; Fasel N
TI Highly inducible synthesis of heterologous proteins in epithelial cells

carrying a glucocorticoid-responsive vector.
SO Gene, (1992 Feb 15) Vol. 111, No. 2, pp. 199-206.
Journal code: 7706761. ISSN: 0378-1119.

L4 ANSWER 71 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
AU Wang, Qingping; Maher, Veronica M.; McCormick, J. Justin
TI Mammalian expression vectors with modulatable promoters and two multiple cloning sites
SO Gene (1992), 119(2), 155-61
CODEN: GENED6; ISSN: 0378-1119

L4 ANSWER 72 OF 87 MEDLINE on STN DUPLICATE 20
AU Vickers T; Baker B F; Cook P D; Zounes M; Buckheit R W Jr; Germany J; Ecker D J
TI Inhibition of HIV-LTR gene expression by oligonucleotides targeted to the TAR element.
SO Nucleic acids research, (1991 Jun 25) Vol. 19, No. 12, pp. 3359-68.
Journal code: 0411011. ISSN: 0305-1048.

L4 ANSWER 73 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
AU De Benedetti, Arrigo; Rhoads, Robert E.
TI A novel BK virus-based episomal vector for expression of foreign genes in mammalian cells
SO Nucleic Acids Research (1991), 19(8), 1925-31
CODEN: NARHAD; ISSN: 0305-1048

L4 ANSWER 74 OF 87 MEDLINE on STN DUPLICATE 21
AU Ebert K M; Selgrath J P; DiTullio P; Denman J; Smith T E; Memon M A; Schindler J E; Monastersky G M; Vitale J A; Gordon K
TI Transgenic production of a variant of human tissue-type plasminogen activator in goat milk: generation of transgenic goats and analysis of expression.
SO Bio/technology (Nature Publishing Company), (1991 Sep) Vol. 9, No. 9, pp. 835-8.
Journal code: 8309273. ISSN: 0733-222X.

L4 ANSWER 75 OF 87 MEDLINE on STN DUPLICATE 22
AU Soifer D; Nicoletti V; Cabane K; Mack K; Poulos B
TI Expression of the neurofilament protein NF-H in L cells.
SO Journal of neuroscience research, (1991 Sep) Vol. 30, No. 1, pp. 63-71.
Journal code: 7600111. ISSN: 0360-4012.

L4 ANSWER 76 OF 87 MEDLINE on STN DUPLICATE 23
AU Quarmby V E; Kemppainen J A; Sar M; Lubahn D B; French F S; Wilson E M
TI Expression of recombinant androgen receptor in cultured mammalian cells.
SO Molecular endocrinology (Baltimore, Md.), (1990 Sep) Vol. 4, No. 9, pp. 1399-407.
Journal code: 8801431. ISSN: 0888-8809.

L4 ANSWER 77 OF 87 LIFESCI COPYRIGHT 2006 CSA on STN
AU Jacquemin-Sablon, H.; Bogenberger, J.
TI Glucocorticoids regulate expression of the v-myc super(O) super(K) super(1) super(0) oncogene in a murine retroviral vector with chimeric MoMuLV-MMTV LTRs.
SO BIOCHEM. INT., (1990) vol. 20, no. 4, pp. 669-679.

L4 ANSWER 78 OF 87 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
AU PUGA A [Reprint author]; RAYCHAUDHURI B; SALATA K; ZHANG Y-H; NEBERT D W
TI STABLE EXPRESSION OF MOUSE CYP1A1 AND HUMAN CYP1A2 COMPLEMENTARY DNA TRANSFECTED INTO MOUSE HEPATOMA CELLS LACKING DETECTABLE P-450 ENZYME ACTIVITY.
SO DNA and Cell Biology, (1990) Vol. 9, No. 6, pp. 425-436.
CODEN: DCEBE8. ISSN: 1044-5498.

L4 ANSWER 79 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
AU Johansen, Teit Eliot; Schoeller, Marianne Skak; Tolstoy, Susanne; Schwartz, Thue W.
TI Biosynthesis of peptide precursors and protease inhibitors using new constitutive and inducible eukaryotic expression vectors
SO FEBS Letters (1990), 267(2), 289-94
CODEN: FEBLAL; ISSN: 0014-5793

L4 ANSWER 80 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
AU Ko, Minoru S. H.; Takahashi, Naomi; Sugiyama, Norifumi; Takano, Toshiya
TI An auto-inducible vector conferring high glucocorticoid inducibility upon stable transformant cells
SO Gene (1989), 84(2), 383-9
CODEN: GENED6; ISSN: 0378-1119

L4 ANSWER 81 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
IN Okano, Kiyoshi; Sawada, Ritsuko; Shimizu, Hirohiko
TI Interferons and their manufacture with recombinant animal cells
SO Jpn. Kokai Tokkyo Koho, 17 pp.
CODEN: JKXXAF

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 63141588	A2	19880614	JP 1986-288880	19861205

L4 ANSWER 82 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
IN Okano, Kiyoshi; Sawada, Ritsuko; Shimizu, Hirohiko
TI Human lung cancer cells for manufacture of polypeptides
SO Jpn. Kokai Tokkyo Koho, 21 pp.
CODEN: JKXXAF

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 63141582	A2	19880614	JP 1986-288878	19861205
JP 2530631	B2	19960904		

L4 ANSWER 83 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
IN Okano, Kiyoshi; Sawada, Ritsuko; Shimizu, Hirohiko
TI Glycopeptides and their recombinant production with human cells
SO Jpn. Kokai Tokkyo Koho, 11 pp.
CODEN: JKXXAF

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 63141581	A2	19880614	JP 1986-288877	19861205

L4 ANSWER 84 OF 87 LIFESCI COPYRIGHT 2006 CSA on STN
AU van Klaveren, P.; Bentvelzen, P.
TI Transactivating potential of the 3' open reading frame of murine mammary tumor virus.
SO J. VIROL., (1988) vol. 62, no. 11, pp. 4410-4413.

L4 ANSWER 85 OF 87 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
AU SU H K [Reprint author]; COURTNEY R J
TI INDUCIBLE EXPRESSION OF HERPES SIMPLEX VIRUS TYPE 2 GLYCOPROTEIN GENE GG-2 IN A MAMMALIAN CELL LINE.
SO Journal of Virology, (1988) Vol. 62, No. 10, pp. 3668-3674.
CODEN: JOVIAM. ISSN: 0022-538X.

L4 ANSWER 86 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
AU Cato, A. C. B.; Skroch, P.; Weinmann, J.; Butkeraitis, P.; Ponta, H.
TI DNA sequences outside the receptor-binding sites differentially modulate the responsiveness of the mouse mammary tumor virus promoter to various steroid hormones
SO EMBO Journal (1988), 7(5), 1403-10

CODEN: EMJODG; ISSN: 0261-4189

L4 ANSWER 87 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
AU Matthias, Patrick; Boeger, Uta; Danesch, Ulrich; Schutz, Guenther; Bernard, Hans Ulrich
TI Physical state, expression and regulation of two glucocorticoid-controlled genes on bovine papilloma virus vectors
SO Journal of Molecular Biology (1986), 187(4), 557-68
CODEN: JMOBAK; ISSN: 0022-2836

=> d ab 62-80 l4

L4 ANSWER 62 OF 87 MEDLINE on STN DUPLICATE 11
AB To study the influence of various transactivators and the role of nucleosomal structure in gene regulation by steroid hormones, we have introduced **mouse mammary tumor virus** (**MMTV**) **promoter** sequences along with expression **vectors** for the glucocorticoid receptor (GR) and nuclear factor I (NFI) in Saccharomyces cerevisiae, an organism amenable to genetic manipulation. Both in the context of an episomal multicopy vector and in a centromeric single-copy plasmid, the MMTV promoter was virtually silent in the absence of inducer, even in yeast strains expressing GR and NFI. Induction was optimal with deacylcortivazol and required both GR and NFI. The transactivation function AF1 in the N-terminal half of GR is required for ligand-dependent induction and acts constitutively in truncated GR lacking the ligand binding domain. A piece of the MMTV long terminal repeat extending from -236 to +111 is sufficient to position a nucleosome, B, over the regulatory region of the promoter from -45 to -190 and another nucleosome over the transcription start region. The rotational orientation of the DNA on the surface of nucleosome B is the same as that previously found in animal cells and in reconstitution experiments. This orientation is compatible with binding of GR to two sites, while it should preclude binding of NFI and hence be responsible for constitutive repression. Upon ligand induction, there is no major chromatin rearrangement, but the proximal linker DNA, including the TATA box, becomes hypersensitive to nucleases. The transcriptional behavior of the MMTV promoter was unaffected by deletions of the genes for zuotin or SIN1/SPT2, two proteins which have been claimed to assume some of the functions of linker histones. Thus, despite the lack of histone H1, yeast cells could be a suitable system to study the contribution of nucleosomal organization to the regulated expression of the MMTV promoter.

L4 ANSWER 63 OF 87 MEDLINE on STN DUPLICATE 12
AB Genetic alteration of the set of oligosaccharide biosynthesis enzymes expressed in a genetically engineered host cell line is a plausible strategy for manipulating the oligosaccharides on a cloned glycoprotein coexpressed in that cell line. This hypothesis was verified for the particular case of sialylation of recombinant human tissue plasminogen activator (tPA) expressed by an engineered Chinese hamster ovary (CHO) cell line. The gene for rat liver beta-galactoside alpha(2,6)-sialyltransferase (2,6-ST) was cloned behind the **MMTV** **promoter** in the **vector** pMSG and transfected into a tPA-expressing CHO cell line. Selected and screened transfectants exhibited significantly greater surface fluorescence than controls in flow cytometric analyses of cells labeled with Sambacus nigra agglutinin (SNA)-biotin and streptavidin-R-phycoerythrin; SNA specifically binds to NeuAc alpha(2,6)Gal beta(1,4)Glc-N-AcR linkages, which are synthesized by 2,6-ST and which are not normally found on CHO cells. SNA blots of partially purified tPA from the culture supernatant demonstrated that tPA synthesized in the 2,6-ST transfectants possessed terminal NeuAc alpha(2,6)Gal beta(1,4)Glc-N-AcR linkages, while tPA from the original recombinant CHO cell line did not. Besides possibly allowing the production of glycoproteins in cell culture with glycosylation more

closely resembling that in humans, extensions of this strategy have the potential to tailor the pharmacokinetics, targeting, and antigenic properties of cloned glycoproteins.

L4 ANSWER 64 OF 87 MEDLINE on STN DUPLICATE 13
AB Two sets of eucaryotic expression vectors encoding trans-acting hammerhead ribozymes and trans-acting hairpin ribozymes were constructed. In one set of vectors ribozyme RNA transcription was placed under the control of a mouse mammary tumor virus long terminal repeat (MMTV-LTR). In the other set ribozyme expression was controlled by a metallothionein IIA (Mt-IIA) promoter. Each ribozyme was directed to the first target sequence in the Alzheimer amyloid peptide precursor mRNA (beta APP mRNA), 5' decreases GUC decreases 3'. Ribozyme RNA transcribed from these vectors, which should cleave all six alternatively spliced forms of beta APP mRNA as well as beta APP pre-mRNA, was shown to cleave a beta APP RNA substrate analog in vitro. Stably transfected COS-7 cell lines bearing both vector types were prepared. Steady-state levels of beta APP mRNA were reduced 25-30% in cells containing either active or mutant hammerhead ribozyme **vectors** driven by the **MMTV**-LTR **promoter** grown in the presence of glucocorticoids. In cell lines bearing Mt-IIA driven ribozymes steady-state levels of beta APP mRNA were reduced 67-80% in both hammerhead and hairpin ribozyme containing cell lines following promoter induction by glucocorticoids. These levels correlate with the appearance of low levels of induced ribozyme RNA. In contrast, steady-state alpha-actin mRNA and G3PDH mRNA levels in these cells remained constant. Western blotting of cell extracts revealed that all forms of beta APP were correspondingly reduced. Neither the RNA nor protein decreases observed in ribozyme transfected cell lines were observed in stably transfected control cells bearing the vector alone. These results suggest that ribozyme-mediated degradation of beta APP mRNA in COS-7 cells does not depend on ribozyme cleavage.

L4 ANSWER 65 OF 87 MEDLINE on STN DUPLICATE 14
AB Alterations in the amino acid composition, phosphorylation pattern, or intracellular levels of topoisomerase II have been associated with resistance to antineoplastic agents whose effects are mediated through interactions with this enzyme. To develop a model system with which to investigate the determinants of topoisomerase II sensitivity or resistance to antineoplastic agents that target this enzyme, a cDNA encoding the wild-type Drosophila melanogaster topoisomerase II was ligated into a mammalian expression **vector** containing a glucocorticoid-inducible **mouse mammary tumor virus promoter** and transfected into an epipodophyllotoxin-resistant Chinese hamster ovary cell line (VPM(r)-5). In two transfectants carrying an intact, full-length Drosophila topoisomerase II cDNA, exposure to the inducing agent, dexamethasone (10 microM), resulted in complementation of the endogenous mutant topoisomerase II and phenotypic reversion to etoposide sensitivity. In the presence of glucocorticoid, etoposide-induced cytotoxicity increased 20-fold, despite the fact that Drosophila topoisomerase II mRNA expression was only 0.1% of that of the endogenous mammalian topoisomerase II. Induced cells demonstrated a marked increase in DNA single strand breaks compared with uninduced resistant cells, thereby providing biochemical evidence supporting increased DNA strand cleavage due to activation of the Drosophila enzyme. These observations demonstrate the ability of a wild-type Drosophila topoisomerase II to complement a mutant mammalian enzyme and suggest that transfectants capable of conditional topoisomerase II expression represent a useful model for studies of the biochemical pharmacology and structure-function relationships of normal and mutant enzymes.

L4 ANSWER 66 OF 87 MEDLINE on STN DUPLICATE 15
AB An M(r) 85,000 membrane protein was identified by a monoclonal antibody MRK20 raised against an Adriamycin-resistant subline of human myelogenous leukemia K562 (K562/ADM) cells. The M(r) 85,000 protein was found to be

overexpressed in both innate and acquired Adriamycin-resistant tumor lines. A complementary DNA (cDNA) clone coding for the M(r) 85,000 protein was isolated by mixed oligonucleotide-primed polymerase chain reaction and further screening of a cDNA library from K562/ADM. Amino acid and nucleotide sequence analysis of the M(r) 85,000 protein revealed that this protein is identical with CD36, a cell surface adhesion molecule of endothelium, platelets, and monocytes. We constructed an expression **vector** utilizing two different **promoters**, SV40 and **MMTV**, and two cDNAs for the M(r) 85,000 protein that have different 3'-ends. DNA transfection experiments were carried out by the calcium phosphate method with a selectable marker using drug-sensitive human tumor lines KB3-1 and A2780 as recipient cells. We obtained transfectant clones expressing the M(r) 85,000 protein stably or inducibly but found no resistance against Adriamycin or vincristine. Direct selection with Adriamycin or vincristine or tumor cells transfected with the SV40 promoter-regulated expression constructs also failed to yield drug-resistant clones. These results indicate that the M(r) 85,000 protein/CD36 cannot confer drug resistance by itself, even though the protein can be an effective marker for Adriamycin resistance.

L4 ANSWER 67 OF 87 MEDLINE on STN DUPLICATE 16

AB The pathogenicity of the human c-erbB-2 oncogene was evaluated in transgenic mice. A **DNA sequence** comprising the **promoter**-enhancer region of the **MMTV** LTR and a constitutively activated allele of the human c-erbB-2 growth factor receptor gene was introduced into the germ line of mice. Expression of the transgene was observed in kidney, lung, mammary gland, salivary gland, Harderian gland, and in epithelial cells of the male reproductive tract. All transgenic mice expressing the c-erbB-2 receptor died within four months of birth. Histopathological analysis suggests that preneoplastic lesions in kidney and lung most likely caused organ failure and the early death of the transgenic mice. Focal dilatation and atypical proliferation of the tubular epithelial cells was found in the kidney. These hyperplastic lesions were found adjacent to normal tubules. Immunohistochemistry showed that normal renal structures were completely negative for c-erbB-2 protein expression. Atypical pseudopapillary proliferation of bronchial and bronchiolar epithelial cells narrowed the bronchial lumen in lung. Alveoli appeared normal. The expression of c-erbB-2 protein was strictly limited to the proliferating epithelial cells and not detected in normal tissue. The mammary glands of two parous mice were underdeveloped, lacking lobular-alveolar structures and were lactation deficient. Only a few ducts were interspersed in the fat pad. A virgin mouse developed a focal adenocarcinoma infiltrating the mammary fat pad. Expression of the c-erbB-2 protein was enhanced in the proliferating epithelial cells. Transgenic males were sterile. Epithelial hyperplasia and hypertrophy in the epididymis, vas deferens and seminal vesicles was found. The transgene is not uniformly expressed in the tissues where the MMTV LTR is transcriptionally active. The scattered transgene expression invariably coincides with epithelial hyperplasia.

L4 ANSWER 68 OF 87 MEDLINE on STN DUPLICATE 17

AB Irs1 cells are mutants of the Chinese hamster cell line V79-4, and exhibit cross-sensitivity to various DNA-damaging agents, especially to the alkylating compounds methyl methanesulfonate and ethyl methanesulfonate. To test whether this sensitivity was due to the persistence of alkylated residues in DNA, we have transfected irs1 cells with the pMSG expression vector containing two coding sequences for enzymes of different origin, either the E. coli AlkA gene, coding for 3-methyladenine-DNA-glycosylase II, or rat APDG cDNA, encoding alkylpurine-DNA-glycosylase. The two coding sequences for the repair enzymes were ligated in the pMSG **vector**, under the control of the **MMTV**-LTR **promoter**, which is responsive to glucocorticoid regulation. The presence of the AlkA gene or of the APDG cDNA in the transfected cells was detected by Southern blot analysis and the transcription of these foreign

sequences was checked by Northern hybridization of the cellular RNA. The transfected irs1 cells treated with [3H]dimethylsulfate removed the 3-methyladenine residues more efficiently from their DNA than the control cells. Irs1 cells harboring the AlkA or the APDG gene become about 2- and 3-fold more resistant to the toxic effect of methyl methanesulfonate, respectively. However, a 3-fold resistance to ethyl methanesulfonate was only observed in irs1 cells harboring the mammalian APDG cDNA.

L4 ANSWER 69 OF 87 MEDLINE on STN DUPLICATE 18
AB A full-length Na+/glucose cotransporter cDNA (SGLT1) from rabbit intestine was subcloned into the pMAMneo mammalian expression vector and transfected by Ca2+ precipitation into Madin-Darby canine kidney (MDCK) cells. Stable MDCK transfectants isolated after clonal isolation and selection in G418 exhibited dexamethasone-inducible Na+/glucose cotransport activity under regulation of the **MMTV promoter** of the **vector** . Transfectants expressed the recombinant 75 kDa Na+/glucose cotransporter subunit as shown by Western blot, and SGLT1 mRNA as shown by Northern blot, but these were undetectable in untransfected MDCK cells. Over 93% of total recombinant transport activity was targeted to the apical membrane. This indicates that the primary amino acid sequence of SGLT1 contains the information necessary to target this transporter to the apical membrane.

L4 ANSWER 70 OF 87 MEDLINE on STN DUPLICATE 19
AB A glucocorticoid-responsive vector is described which allows for the highly inducible expression of complementary DNAs (cDNAs) in stably transfected mammalian cell lines. This **vector**, pLK-neo, composed of a variant **mouse mammary tumor virus** long terminal repeat **promoter**, containing a hormone regulatory element, a Geneticin resistance-encoding gene in a simian virus 40 transcription unit, and a polylinker insertion site for heterologous cDNAs, was used to express the polymeric immunoglobulin (poly-Ig) receptor and the thymocyte marker, Thy-1, in Madin-Darby canine kidney (MDCK) cells and in murine fibroblast L cells. A high level of poly-Ig receptor or Thy-1 mRNA accumulation was observed in MDCK cells in response to dexamethasone with a parallel ten- to 200-fold increase in protein synthesis depending on the recombinant protein and the transfected cell clone.

L4 ANSWER 71 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
AB To facilitate the use of a wide range of selectable markers in transfection studies with human cells, in conjunction with the use of modulatable promoters for regulated expression of the genes of interest, two pUC19-based mammalian expression vectors, each containing two lacZα-based multiple cloning sites (MCS) were constructed. Selectable markers can be inserted into the MCS derived from pUC19, and the recombinants can be screened by lacZ complementation. The genes of interest can be inserted into the second MCS. The new MCS contains an amber stop codon in-frame with translation of the LacZ α-peptide. The presence of insert in the second MCS can also be screened on XGal plates, but in an Escherichia coli host containing an amber suppressor gene. Expression of the genes of interest can be modulated through transcription from the promoter of the mouse metallothionein-I-encoding gene or the long terminal repeat of the mouse mammary tumor virus. These vectors, as well as several of the intermediate plasmids described in this report, can be used to clone any two genetic elements into a single plasmid.

L4 ANSWER 72 OF 87 MEDLINE on STN DUPLICATE 20
AB All human immunodeficiency virus mRNAs contain a sequence known as TAR (trans-activating responsive sequence). The TAR element forms a stable RNA stem-loop structure which binds the HIV tat (trans-activator) protein and mediates increased viral gene expression. In principle, molecules which bind to the TAR RNA structure would inhibit trans-activation by perturbing the native RNA secondary structure. We have constructed a

series of phosphodiester and phosphorothioate antisense oligonucleotides which specifically bind to the HIV TAR element. Specific binding to the TAR element was demonstrated in vitro with enzymatically synthesized TAR RNA. The TAR-directed phosphorothioates inhibited trans-activation in a sequence-dependent fashion in a cell culture model using an HIV LTR/human placental alkaline phosphatase gene fusion and tat protein supplied in trans. The molecules also inhibited HIV replication in both acute and chronically infected viral assays, but without sequence specificity. We have constructed a series of **vectors** consisting of the **MMTV promoter** and 5'-untranslated region of four different mRNAs, including the TAR region, to study the effect of TAR on gene expression in heterologous systems. The results suggest that, in the absence of the HIV LTR, the TAR element has a repressive effect on gene expression, which is relieved by tat.

L4 ANSWER 73 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN
AB A composite mammalian cell-E. coli shuttle vector was developed based on the human papova virus BK and pSV-neo. The vector contains a dioxin-responsive enhancer (DRE) controlling a mouse mammary tumor virus (MMTV) promoter for the inducible expression of inserted genes. In human cells the vector replicates episomally, presumably utilizing the BKV rather than the SV40 origin, and expresses the BK T/t antigens. A deletion in the late BK region precludes the expression of the core/capsid proteins VP1, VP2, and VP3, thereby preventing the infectious lytic cycle. HeLa cells which were transfected with this vector and selected for resistance to the antibiotic G418 maintained the construct primarily in episomal form during more than one year of continuous culture, with little or no integration into the host genome. Transformed cells cultured in higher concns. of G418 contained higher copy nos. of the vector. This permits one to vary the dosage of an inserted gene easily and reversibly without the need of conventional amplification techniques and clonal anal. Using a chloramphenicol acetyl transferase (CAT) reporter gene inserted downstream of the MMTV promoter, the authors found that CAT expression was greater in clones with higher vector copy number CAT expression was inducible with 2,3,7,8-tetrachlorodibenzo-p-dioxin, but inducibility was found to be inversely proportional to the copy number Transformation of bacteria with plasmid mols. retrieved from the mammalian host was efficient, making this vector well adapted for the screening of cDNA libraries for the ability to express a phenotype in mammalian cells. Moreover, DNA sequences were stable during long-term passage in mammalian cells; vector passaged continuously for more than one year retained fully functional bacterial genes for resistance to chloramphenicol and ampicillin.

L4 ANSWER 74 OF 87 MEDLINE on STN DUPLICATE 21
AB We report the first successful production of transgenic goats that express a heterologous protein in their milk. The production of a glycosylation variant of human tPA (LAtPA--longer acting tissue plasminogen activator) from an expression **vector** containing the murine whey acid **promoter** (**WAP**) operatively linked to the cDNA of a modified version of human tPA was examined in transgenic dairy goats. Two transgenic goats were identified from 29 animals born. The first animal, a female, was mated and allowed to carry the pregnancy to term. Milk was obtained upon parturition and was shown to contain enzymatically active LAtPA at a concentration of 3 micrograms/ml.

L4 ANSWER 75 OF 87 MEDLINE on STN DUPLICATE 22
AB We have inserted a Not1-Sal1 fragment of the mouse gene coding for the neurofilament protein NF-H behind the dexamethasone-inducible transcription **promoter** of **MMTV** in a **vector** derived from pMAMneo (Clonetech Labs). This construct, which includes all four exons of the NF-H gene, was amplified and incorporated into liposomes for transfection of L cells. Transfectants were selected in G418-containing medium and cloned. Clones were grown in serum-containing

medium and screened for expression of the NF-H mRNA by extraction of total RNA, generation of cDNAs by reverse transcription, and amplification of a 900-base portion of the NF-H cDNA by PCR. Positive clones were detected by the presence of a band of the correct size on agarose gels. This was confirmed by Southern blotting of the gels probed with a 185-base segment of the amplified region. Immunofluorescent analysis of two positive clones, C33 and C34, showed that C33 cells grown in serum-containing medium or in serum-free medium in the presence of dexamethasone have a network of SMI32 (Sternberger/Meyer Inc.--monoclonal antibody against a nonphosphorylated epitope on NF-H)-positive filaments with the same distribution as filaments stained with antibodies to vimentin, while C34 cells do not react with antibodies against neurofilament proteins. Neither clone reacted with antibodies against highly phosphorylated NF-H (SMI31).

L4 ANSWER 76 OF 87 MEDLINE on STN DUPLICATE 23

AB Full-length rat and human androgen receptor (AR) cDNA clones were expressed in COS-7 and CV1 monkey kidney cells to analyze the AR protein using immunological and cotransfection techniques. The studies were aided by the development of two rabbit polyclonal antibodies, designated AR32 and AR52, directed against epitopes within the N-terminal region of AR. Each antibody recognizes native AR by sucrose gradient analysis and detects a 114-kilodalton protein in COS cells transfected with human or rat AR cDNA. Covalent binding of the synthetic androgen [3H]methyltrienolone (R1881) to the 114-kDa protein was saturable. The endogenous native AR was similarly 114 kDa on immunoblots of a human prostate adenocarcinoma cell line, LNCaP, and rat sex accessory gland extracts. AR was localized in nuclei of transfected COS cells and in LNCaP cells by immunocytochemical staining. Androgen induction of CAT activity was dose dependent in CV1 cells cotransfected with the AR expression **vector** and a reporter plasmid containing the **mouse mammary tumor virus** **promoter** linked to the chloramphenicol acetyltransferase gene. It is concluded that antipeptide antibodies are useful reagents in characterizing both native and denatured forms of the AR protein. The 114-kDa protein expressed transiently in cultured cells represents the full-length AR protein, has a molecular size equivalent to that of endogenous AR, and mediates androgen-dependent transcriptional activation in CV1 cells.

L4 ANSWER 77 OF 87 LIFESCI COPYRIGHT 2006 CSA on STN

AB A murine retrovirus which expresses the v-myc super(O) super(K) super(1) super(0) oncogene under the control of the dexamethasone-regulatable **mouse mammary tumor virus** (**MMTV**) **promoter** has been constructed. In this **vector**, denoted pMImyc, the Moloney murine leukemia virus (MoMuLV) sequences required for virus replication, integration and packaging were kept, while all the elements for transcription regulation were derived from the MMTV long terminal repeat (LTR). After transfection of NIH 3T3 fibroblasts with this construct, a cell line was isolated in which the level of v-myc RNAs were increased 60 fold by dexamethasone.

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AB Using the mouse hepatoma Hepa-1c1c7 c37 mutant cell line that exhibits negligible benzo[α]pyrene hydroxylase (Cyp1a1) and acetanilide 4-hydroxylase (Cyp1a2) enzyme activities, we developed stable transfectants of plasmids containing the murine Cyp1a1 (cytochrome P1 450) and the human CYP1A2 (P3 450) cDNAs. We show that the assay measuring metabolism of ethoxyfluorescein ethyl ester (EFEE) was invaluable in screening large numbers of individual cell lines for high Cyp1a1 enzyme activity. Nine different plasmid constructs containing various combinations of promoter and enhancer sequences were compared, including:the Drosophila heat shock promoter, the mouse mammary tumor

virus long terminal repeat (MMTV LTR) carry in the glucocorticoid-responsive element (GRE), enhancer sequences from simina virus 40 (SV40) and herpes simplex virus type 1 (HSV-1), and the aromatic hydrocarbon-responsive domain (AhRD) of the murine Cyp1a1 gene. Interestingly, only those constructs containing the AhRD produced high levels of Cyp1a1 enzyme activity. In contrast, high levels of CYP1A2 activity were obtained with plasmids carrying the HSV-1 enhancer, as well as the AhRD. These studies suggest that the AhRD, which responds to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), provides a post-transcriptional signal necessary for the induction of functional Cyp1a1 enzyme activity. Although untransfected c37 cells exhibit markedly elevated levels of endogenous Cyp1a1 mRNA, the expression of exogenous Cyp1a1 or CYP1A2 enzyme might have a role in an autoregulatory loop controlling the constitutive expression of the Cyp1a1 gene. The cell lines described here in should be valuable in assessing the contribution of these two P450 enzymes to the processes of cytotoxicity, mutagenesis, and carcinogenesis.

L4 ANSWER 79 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN

AB A series of expression vectors has been constructed based on the pML derivative of pBR322. The eukaryotic transcription units employed various promoters followed by polycloning sites for 3-9 commonly used restriction enzymes and are completed by the SV40 polyadenylation sequence. In 4 of the vectors, designed for co-transfection or transient expression studies, only a single transcription unit containing either a constitutive or an inducible promoter was incorporated. The human ubiquitin (UbC) promoter was used as a strong constitutive promoter, while the mouse metallothionein promoter and the promoter of the long terminal repeats of the mouse mammary tumor virus were used as inducible promoters. Another vector contained an addnl. transcription unit encoding a eukaryotic selection marker, the neomycin resistance encoding gene. The vectors were used in CHO cells and in neuroendocrine CA77 cells to synthesize peptide precursors, protease inhibitors and a protease. It is shown that these vectors are very efficient for the constitutive and inducible expression of nucleotide sequences in both transient and stable transfections of eukaryotic cells.

L4 ANSWER 80 OF 87 CAPLUS COPYRIGHT 2006 ACS on STN

AB A new gene expression system in mammalian cells was developed by using the glucocorticoid receptor (GR) as an inducible pos. feedback factor. Mouse Ltk- cells were transfected with plasmids carrying the GR-encoding gene and the lacZ receptor gene, both of which were fused with the glucocorticoid-inducible enhancer/promoter of the mouse mammary tumor virus (MTV). The GR gene was first induced to supply the receptor protein, which further induced the expression of both GR and reporter genes. Stable transformants induced with dexamethasone, a synthetic glucocorticoid hormone, demonstrated β-galactosidase activity 60-140-fold higher than uninduced controls. Similarly, the human α-interferon-encoding gene fused with the MTV enhancer/promoter was induced more than 12,000-fold. This system permitted an increase in the expression of the reporter or target genes without augmenting basal levels of expression significantly, and may be useful to investigate the unknown function of a cloned gene, particularly when the gene product of interest is cytotoxic or growth-inhibiting.